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Influence of Inorganic Phosphate on Photosynthesis of Wheat Chloroplasts

I. PHOTOSYNTHESIS AND ASSIMILATE EXPORT AT 5 °C AND 25 °C

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ABSTRACT

Chloroplasts were isolated from 10 d old wheat seedlings and illuminated at 5 °C or 25 °C in various concentrations of PO_4^{3-}. Photosynthetic oxygen evolution, ATP content, and export of triose phosphates and 3-phosphoglycerate were measured. Incorporation of ^{14}C from $NaH^{14}CO_3$ into pentose monophosphates, fructose monophosphate, and glucose monophosphates was determined.

The ATP content in illuminated chloroplasts decreased when the PO_4^{3-} concentration in the medium was low. The ATP content increased when the PO_4^{3-} concentration was increased. A higher PO_4^{3-} concentration in the medium was needed to increase the ATP at 5 °C than at 25 °C. This would suggest that PO_4^{3-} deficiency occurs more readily at low than at high temperatures. More ^{14}C was incorporated into photosynthetic metabolites within the chloroplasts at 5 °C than at 25 °C, indicating decreased assimilate export when the temperature was low. Dihydroxyacetone phosphate was preferentially exported when the PO_4^{3-} concentration enabled a high rate of photosynthesis at 25 °C. However, under conditions of PO_4^{3-} deficiency, either due to low PO_4^{3-} concentration in the medium or due to low temperature, 3-phosphoglycerate was preferred for export.

The results suggest that the relatively high photosynthetic rates at low temperature are due to increased concentrations of photosynthetic metabolites. The assimilate export at low temperature seems to be decreased due to decreased concentrations of dihydroxyacetone phosphate in the stroma. Preferential export of 3-phosphoglycerate at low temperature or at low PO_4^{3-} concentration in the medium may be a consequence of high stromal concentrations of this metabolite. On the other hand, it could also be due to decreased stromal pH.

Key words: Chloroplast; Photosynthesis; Phosphate; Temperature; Translocation; ATP; Wheat.

INTRODUCTION

Assimilate export from chloroplasts is increased by high concentrations of inorganic phosphate (P_i) in the medium due to a strict counterexchange of triose phosphate or 3-phosphoglycerate (3-PGA) against P_i across the inner chloroplast envelope membrane (Heldt and Rapley, 1970). Maximum chloroplast photosynthesis occurs when, at an optimum P_i concentration, export neither limits nor exceeds CO_2 fixation (Flügge, Freisl, and Heldt, 1980). Low P_i concentration leads to a decrease in assimilate export and an increase in starch synthesis (Heldt, Chon, Maronde, Herold, Stankovic, Walker, Kraminer, Kirk, and Heber,

Abbreviations: P_i: inorganic phosphate; 3-PGA: 3-phosphoglycerate; DHAP: dihydroxyacetone phosphate; ATP: adenosine triphosphate.

1977), whereas at high P_i concentration chloroplasts are depleted of photosynthetic metabolites (Flügge *et al.*, 1980).

Decrease in temperature leads to a decrease in chloroplast photosynthesis and a longer period before photosynthesis reaches its maximum rate following illumination (Walker, Kosciukiewicz, and Case, 1973). The temperature dependence of chloroplast photosynthesis deviates from the Arrhenius law showing an increased activation energy at low temperature. Baldry, Bucke, and Walker (1966) suggested that this is a consequence of the cyclic nature of the Benson–Calvin cycle. Phosphate transport, on the other hand, shows a linear Arrhenius plot with a relatively low activation energy of 16 kcal mol^{-1} (Fliege, Flügge, Werdan, and Heldt, 1978). This disparity between the temperature dependence of photosynthesis and of phosphate translocation does not lead to a depletion of stromal metabolites at low temperature, since a higher P_i concentration is needed for assimilate export at low temperature (Leegood and Walker, 1983).

The present study investigates the influence of temperature and P_i concentration on the contents of photosynthetic metabolites and of ATP within the chloroplasts and on assimilate export, and tries to explain some of the anomalies of the temperature dependence of photosynthesis.

MATERIALS AND METHODS

Seedlings of *Triticum aestivum* L. (cv. Kolibri) were grown in soil for 10 d in growth chambers (PGV-36, Conviron, Winnipeg) at 20 °C/16 °C day/night temperatures and a 16 h photoperiod with a photon flux density of 400 μmol m^{-2} s^{-1} at the soil surface. Chloroplasts were isolated according to the method of Edwards, Robinson, Tyler, and Walker (1978). Photosynthesis experiments were conducted in a medium consisting of 50 mM Hepes KOH (pH 7·6), 0·4 M sorbitol, 1·0 mM EDTA, 1·0 mM $NaHCO_3$ and 200 units catalase per cm^3. At the end of the experiments $NaHCO_3$ concentration was still as high as 0·7 mM. This suboptimal $NaHCO_3$ concentration was chosen in order to have similar conditions as in a concurrent study on regulation of ribulose bisphosphate carboxylase and despite complications due to glycolate export (Usuda and Edwards, 1982). Experiments were performed using the oxygen electrode system (Hansatech Ltd., Hardwick Industrial Estate, Kings Lynn, Norfolk) described by Delieu and Walker (1972). Photon flux density in the cuvette was adjusted to 300 μmol m^{-2} s^{-1}. Temperature was kept at 5 °C or 25 °C using a circulating water bath. ATP was determined by the luciferase method using the luminometer and assay chemicals from LKB Wallac (Turku, Finland). Dihydroxyacetone phosphate and 3-PGA were determined according to Czok (1974). Chromatography and determination of ^{14}C labelled compounds were performed as described earlier (Mächler, Nösberger, and Erismann, 1977).

RESULTS

1. Photosynthesis and ATP content

Chloroplasts were pre-incubated in the dark at various P_i concentrations for 20 min at 25 °C or for 40 min at 5 °C. Suspensions were illuminated and O_2 release recorded. Samples were taken at intervals for determination of ATP.

The time course of photosynthesis was strongly influenced by temperature and P_i concentration due to effects on the photosynthetic rate and on the lag period during induction of photosynthesis (Fig. 1). For better comparison, photosynthetic rate and ATP content were plotted against the sum of O_2 released (Fig. 2).

The ATP content was low in the dark, rose rapidly upon illumination, and decreased again when photosynthesis started after the induction period. There was a sharp decrease in ATP at both temperatures if P_i was not added to the medium, obviously due to rapid decrease in stromal P_i which is a substrate of ATP synthesis. The decrease was less if the P_i concentration was increased. More P_i was needed to relieve the decrease in ATP at 5 °C than at 25 °C. There was no decrease in ATP at 25 °C if 0·6 mM P_i was present, whereas at 5 °C 0·6 mM P_i was insufficient to prevent a decrease in ATP. Photosynthesis was inhibited at 25 °C in

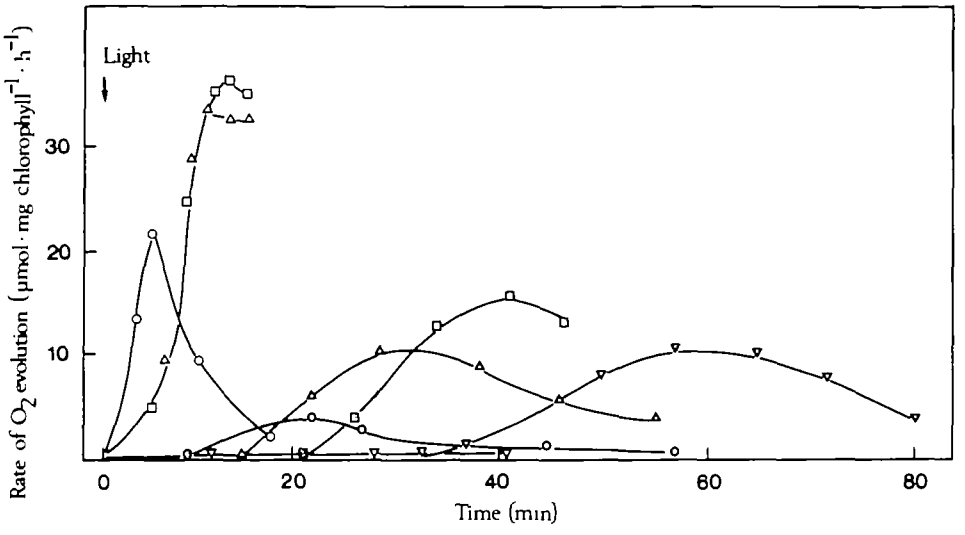

FIG. 1. Induction and rate of photosynthesis by chloroplasts as influenced by temperature and P_i concentration. Chloroplasts (60 μg chlorophyll cm^{-3}) were pre-incubated in the dark for 20 min at 25 °C or for 40 min at 5 °C in various concentrations of P_i with 1·0 mM $NaHCO_3$ and then illuminated. Photosynthetic oxygen release was measured. 25 °C: ○ △ □ ▽, 5 °C: ◒ △ ◨ ▽, no P_i: ○ ◔, 0·2 mM P_i: △ △, 0·6 mM P_i: □ □, 1·8 mM P_i: ▽ ▽.

1·8 mM P_i, probably due to depletion of stromal metabolites. At 5 °C and 1·8 mM P_i the photosynthetic rate was still relatively high and the ATP content decreased as photosynthesis proceeded, indicating that the stroma was not depleted of photosynthetic metabolites.

The results show that phosphate translocation decreases more with temperature than does CO_2 assimilation. The stronger temperature dependence of translocation can be compensated for, at least in part, by an increased P_i concentration at low temperature. The primary consequence of decreased phosphate translocation and P_i deficiency seems to be a decrease in the ATP content.

2. Stromal metabolites

Chloroplasts were illuminated in 2·0 mM $NaH^{14}CO_3$ and 0·3 mM P_i at 5 °C or 25 °C. Samples were taken at intervals and analysed for ^{14}C in pentose monophosphates, fructose monophosphate and glucose monophosphates (Fig. 3). More ^{14}C was found in these compounds when photosynthesis occurred at 5 °C than at 25 °C. At 5 °C the levels of ^{14}C in pentose monophosphates and fructose monophosphate increased first, followed by glucose monophosphates, which are the precursors of starch synthesis. As a control, chloroplasts illuminated at 5 °C were centrifuged for 10 min at 3000 × *g* before analysis: 90% of the labelled compounds mentioned above were found in the chloroplast fraction and only 10% in the supernatant indicating accumulation of these compounds within the chloroplasts. The results support the hypothesis that the balance between CO_2 assimilation and assimilate export is disturbed at low temperature due to decreased phosphate translocation.

3. The exported metabolite

Chloroplast suspensions were illuminated at various P_i concentrations at 5 °C or 25 °C. Suspensions were analysed for dihydroxyacetone phosphate (DHAP), glyceraldehyde phosphate and 3-PGA after 2–3 μmol O_2 had been released per mg chlorophyll because of

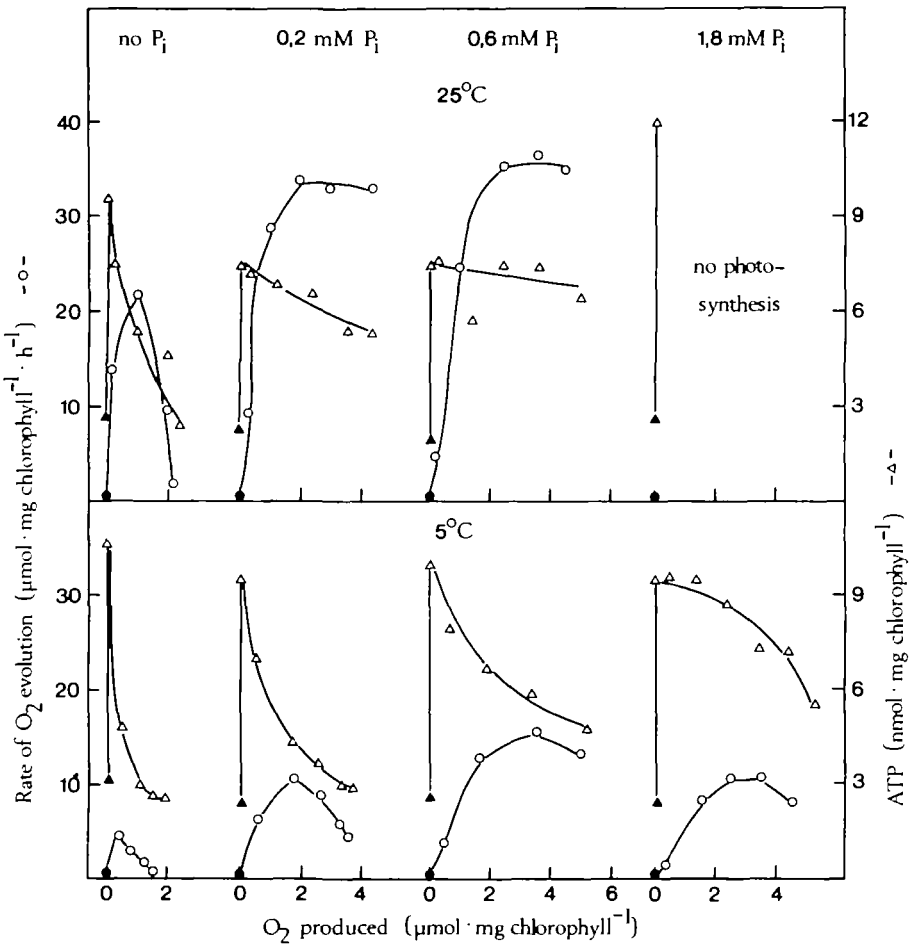

FIG. 2. ATP contents and photosynthesis in chloroplasts as influenced by temperature and P_i concentration. Experimental conditions were the same as in Fig. 1. 100 mm³ samples of the chloroplast suspensions were taken at intervals, mixed immediately with 20 mm³ of 3 N $HClO_4$ and centrifuged for 10 min at 3000 × *g*. 20 mm³ of 1·0 M K_2HPO_4 was added to 100 mm³ of the supernatant and the pH was adjusted to 7·2 by adding 18 mm³ of 3 N KOH. $KClO_4$ was sedimented and ATP in the supernatant determined by the luciferase method. Photosynthetic rates (O) and ATP contents (Δ) are plotted against the sum of O_2 released per mg chlorophyll. Filled symbols indicate measurements made in the dark before illumination.

photosynthesis. Centrifugation of the suspensions before analysis showed that the measured compounds were contained in the supernatant. Concentrations in the chloroplast fraction were too low to be detected by the method used.

DHAP was the preferred metabolite for assimilate export when, at 25 °C and 0·1 mM P_i, photosynthesis seemed to be in good balance with assimilate export (Fig. 2, Table 1). 3-PGA was preferred for export and only a little DHAP was transported when, at low temperature or decreased P_i concentration, the balance between CO_2 assimilation and assimilate export was disturbed due to decreased phosphate translocation. The results indicate that DHAP export is decreased at low temperatures and at 25 °C when P_i concentration is low, whereas a decrease of 3-PGA export does not seem to occur under these conditions.

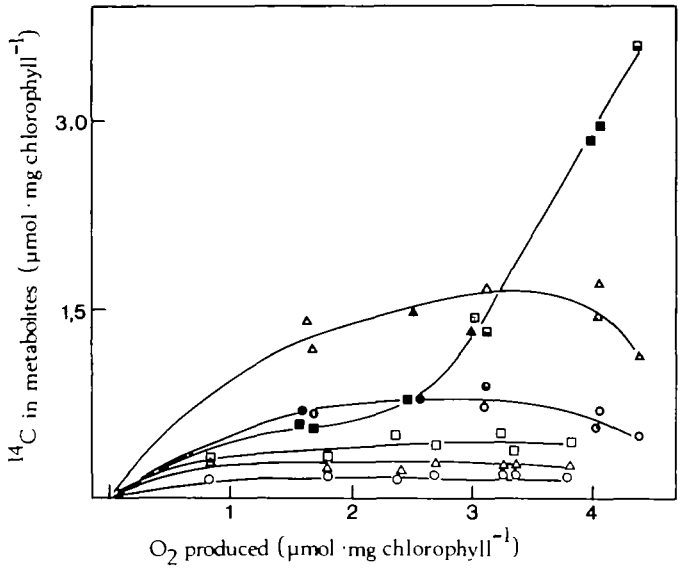

FIG. 3. ^{14}C incorporation into pentose monophosphates, fructose monophosphate and glucose monophosphates during photosynthesis by chloroplasts in the presence of $NaH^{14}CO_3$ at 5 °C and 25 °C. Chloroplasts (65 μg chlorophyll cm^{-3}) were illuminated in the presence of 2·0 mM $NaH^{14}CO_3$ (5·0 Ci mol^{-1}) and 0·3 mM P_i at 5 °C (● ▲ ■) and 25 °C (○ △ □). 100 mm^3 samples were taken at intervals and mixed immediately with 400 mm^3 of methanol. The extracts were separated by thinlayer chromatography and ^{14}C in pentose monophosphates (○ ●), fructose monophosphate (△ ▲), and glucose monophosphates (□ ■) determined.

TABLE 1. *Photosynthesis of DHAP and 3-PGA by chloroplasts illuminated at different temperatures and P_i concentrations*

Chloroplasts (80 μg chlorophyll cm^{-3}) were illuminated at the temperatures and P_i concentrations indicated. Photosynthesis was stopped by adding 200 mm^3 3 N $HClO_4$ to 1·0 cm^3 suspension after about 2·0 μmol O_2 mg^{-1} chlorophyll had been released. The mixture was centrifuged, neutralized and analysed spectrophotometrically for DHAP and 3-PGA according to Czok (1974).

Treatment	μmoles DHAP / μmole O_2 released	μmoles 3-PGA / μmole O_2 released
25 °C, no P_i	0·067	0·156
	0·044	0·152
25 °C, 0·1 mM P_i	0·215	0·063
	0·234	0·054
5 °C, 0·1 mM P_i	0·081	0·123
	0·080	0·122

DISCUSSION

Chloroplast photosynthesis is unexpectedly high at low temperature. The present data suggest that this is due to increased concentrations of photosynthetic metabolites. On the other hand, the activation state of ribulose bisphosphate carboxylase increases as temperature is decreased (Mächler, 1981; Schnyder, Mächler, and Nösberger, 1984), suggesting that high rates at low temperature could also be due to high enzyme activity.

Accumulation of photosynthetic metabolites at low temperature indicates that phosphate translocation through the chloroplast envelope decreases more with temperature than does CO_2 assimilation. This is not due to decreased activity of the phosphate translocator at low temperature, since its activation energy is relatively low (Fliege *et al.*, 1978). The present study shows that at low temperature the transport of DHAP, but not of 3-PGA, is decreased, suggesting that the decreased export is due to decreased stromal concentration of DHAP. DHAP concentration may control assimilate export. A decreased DHAP concentration may prevent chloroplasts from depletion of metabolites during the induction lag of photosynthesis, when CO_2 assimilation is very low. DHAP concentration may be controlled by the enzymes of the Benson–Calvin cycle.

Decreased assimilate export, either due to decreased temperature or due to decreased P_i concentration in the medium, results in accumulation of photosynthetic metabolites, decreased stromal P_i, and a decreased ATP/ADP ratio. ADP inhibits the 3-PGA kinase reaction (Slabas and Walker, 1976) and may cause a decreased DHAP/3-PGA ratio. The preferential export of 3-PGA under these conditions may simply be a consequence of an increased concentration of this metabolite in the stroma. Alternatively, since 3-PGA is transported only as the divalent ion and is present principally as the trivalent ion at physiological pH, it may be that the stromal pH is lower at low temperature and low P_i concentration due to decreased ATP and increased 3-PGA.

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